

**The protozoan parasite *Trichomonas gallinae* causes adult and nestling mortality in a declining population of European Turtle Doves, *Streptopelia turtur*.**

Jennifer E. Stockdale<sup>1,4\*</sup>, Jenny C. Dunn<sup>2\*</sup>, Simon J. Goodman<sup>1</sup>, Antony J. Morris<sup>2</sup>, Danaë K. Sheehan<sup>2</sup>, Philip V. Grice<sup>3</sup> and Keith C. Hamer<sup>1</sup>

1. School of Biology, Irene Manton Building, University of Leeds, Leeds LS2 9JT, UK.

2. RSPB Centre for Conservation Science, Royal Society for the Protection of Birds, The Lodge, Potton Road, Sandy, Bedfordshire SG19 2DL, UK.

3. Natural England, Suite D, Unex House, Bourges Boulevard, Peterborough, PE1 1NG

4. Cardiff School of Biosciences, The Sir Martin Evans Building, Museum Avenue, Cardiff CF10 3AX, UK.

**RUNNING TITLE:** Mortality in European Turtle Doves.

\* Correspondence authors and addresses for correspondence:

Jennifer E. Stockdale,

Cardiff School of Biosciences, The Sir Martin Evans Building, Museum Avenue, Cardiff, CF10 3AX, UK. +44 (0) 2920 875776

StockdaleJE@cardiff.ac.uk

Jenny C. Dunn,

RSPB Centre for Conservation Science, RSPB, The Lodge, Potton Road, Sandy, Bedfordshire, SG19 2DL, UK. +44 (0) 1767 693592

Jenny.Dunn@rspb.org.uk

27 **SUMMARY**

28 Studies incorporating the ecology of clinical and sub-clinical disease in wild populations of  
29 conservation concern are rare. Here we examine sub-clinical infection by *Trichomonas*  
30 *gallinae* in a declining population of free-living European Turtle Doves and suggest caseous  
31 lesions cause mortality in adults and nestlings through subsequent starvation and/or  
32 suffocation. We found a 100% infection rate by *T. gallinae* in adult and nestling Turtle Doves  
33 (n=25) and observed clinical signs in three adults and four nestlings (28%). Adults with  
34 clinical signs displayed no differences in any skeletal measures of size but had a mean 3.7%  
35 reduction in wing length, with no overlap compared to those without clinical signs. We also  
36 identified *T. gallinae* as the suggested cause of mortality in one Red-legged Partridge although  
37 disease presentation was different. A minimum of four strains of *T. gallinae*, characterised at  
38 the ITS/5.8S/ITS2 ribosomal region, were isolated from Turtle Doves. However, all birds with  
39 clinical signs (Turtle Doves and the Red-legged Partridge) carried a single strain of *T. gallinae*,  
40 suggesting that parasite spill over between Columbidae and Galliformes is a possibility that  
41 should be further investigated. Overall, we highlight the importance of monitoring  
42 populations for sub-clinical infection rather than just clinical disease.

43

44 **KEYWORDS:** disease, feeding ecology, supplementary food, necropsy, PCR.

45

46

47

48

49

50

51

52    **KEY FINDINGS**

- 53        • First known cases of mortality in adult and nestling Turtle Doves from trichomonosis.
- 54        • 100% infection rate by *T.gallinae* in Turtle Doves with clinical signs in 28% of birds.
- 55        • Birds with clinical signs had 3.7% shorter wings: no variance in skeletal measures.
- 56        • Parasite spill over potential between Columbidae and Galliformes should be
- 57        investigated.
- 58        • Important to monitor populations for both clinical and sub-clinical infection.

59

## 60 INTRODUCTION

61 The avian disease trichomonosis has a global distribution and widespread infection potential  
62 and is now considered a major contributing factor to the regulation and even decline of avian  
63 populations (Stabler 1954; Krone *et al.* 2005; Forrester and Foster 2008; Robinson *et al.*  
64 2010; Amin *et al.* 2014). In recent years, trichomonosis has undergone a European spread as a  
65 consequence of avian migration from the UK and has been linked to widespread declines in  
66 finch (Fringillidae) populations (Robinson *et al.* 2010; Lawson *et al.* 2011b, 2012; Lehtikoinen  
67 *et al.* 2013; Ganas *et al.* 2014). This recent trichomonosis epizootic reported in finches is  
68 thought to have resulted from parasite spill over of one clonal strain of the *Trichomonas*  
69 *gallinae* parasite from Columbidae to new host species at shared communal garden feeding  
70 stations (Lawson *et al.* 2012; Ganas *et al.* 2014). Within the UK, *T. gallinae* has recently been  
71 reported within four species of Columbidae (Lennon *et al.* 2013).

72

73 Trichomonosis can result in death by suffocation and/or starvation due to caseous  
74 ulcerations/lesions (Stabler 1954). However, host susceptibility and parasite virulence vary,  
75 and hosts often show no clinical signs unless they are nestlings or are infected with a  
76 pathogenic strain (BonDurant and Honigberg 1994; Bunbury *et al.* 2008b; Sansano-Maestre *et*  
77 *al.* 2009; Robinson *et al.* 2010). The trichomonad life cycle has no intermediate host and  
78 transmission can occur horizontally through mutual courtship feeding, or vertically via  
79 transfer of crop milk from adults to nestlings, as well as indirectly through shared food and  
80 water sources (Stabler 1954; Kocan 1969).

81

82 The European Turtle Dove *Streptopelia turtur* (hereafter referred to as ‘Turtle Dove’) is a  
83 trans-Saharan migrant, the populations of which have undergone sustained declines in  
84 abundance and contractions in range. At a pan-European level, Turtle Doves declined by 73%

85 between 1980 and 2010 (PECBMS 2012). In the UK, declines of 93% were recorded between  
86 1970 and 2010 (Eaton *et al.* 2012), with a coinciding 51% reduction in range (Balmer *et al.*  
87 2013).

88

89 Turtle Dove population declines on UK breeding grounds have been attributed to a reduction  
90 in breeding productivity (Browne and Aebischer 2004), alongside a concurrent dietary switch  
91 from 'natural' arable plant seeds to anthropogenic food resources such as grain piles in  
92 farmyards (Browne and Aebischer 2003). The dietary switch and the reduction in breeding  
93 attempts may reflect diminished availability of any food rather than quality alone. This change  
94 in feeding behaviour increases the potential for interactions between the main UK species of  
95 Columbidae and other granivorous farmland birds, including introduced game birds known to  
96 be carriers of *T. gallinae* (Pennycott 1998; Höfle *et al.* 2004).

97

98 Limited information is available about the infection rate of the *T. gallinae* parasite in free-  
99 living Turtle Doves, though Muñoz (1995) found an infection rate of 50% in Spain. Lennon *et*  
100 *al.* (2013) found a high incidence of trichomonad parasite infection (86%) in Turtle Doves on  
101 breeding grounds in the UK; as high as or higher than in any resident species of Columbidae.

102

103 Here we describe mortality in adult and nestling Turtle Doves caused by a single strain of the  
104 protozoan parasite *T. gallinae*, strongly suggested through gross necropsy and subsequent  
105 isolation, culture and sequencing of extracted parasites. We also cultured *T. gallinae* parasites  
106 from artificial food and water sources, suggesting likely routes of transmission.

## 107 MATERIALS AND METHODS

108 Birds were sampled during May – July 2012 on farms in East Anglia, UK at three sites in  
109 Essex (Tolleshunt D'Arcy: 51° 77'N, 0° 79'E; Marks Tey: 51° 88'N, 0° 79'E; and Silver End: 51°  
110 85'N, 0° 62'E) and one in Norfolk (Hilgay: 52° 56'N, 0° 39'E). Sites were baited with either  
111 Wheat *Triticum spp.*, Oil Seed Rape *Brassica napus*, or a standard wild bird seed mix (Maize  
112 *Zea mays L.*, Sunflower *Helianthus annuus*, Pinhead Oatmeal *Avena sativa*, Wheat, Red Dari  
113 *Sorghum L.*, Red and Yellow Millet *Panicum miliaceum*, Hempseed *Cannabis sativa* and Canary  
114 seed *Phalaris canariensis*) in areas where farmers regularly provided supplementary food or  
115 grain tailings, known to be an increasingly important constituent of Turtle Dove diet in the  
116 UK, especially in the early breeding season (Browne and Aebischer 2003). Adults were caught  
117 at each site with either whoosh nets or mist nets (Redfern and Clark 2001). Individuals  
118 displaying clinical symptoms of trichomonosis (feathering around the beak matted, wet and  
119 discoloured by regurgitated saliva) were caught at two of the sites in Essex (Tolleshunt D'Arcy  
120 and Marks Tey), approximately 18 km apart.

121

122 Every bird captured was ringed with a British Trust for Ornithology (BTO) individually  
123 numbered leg ring, weighed with a digital balance (Satrue, Taiwan,  $\pm 0.1$ g) and standard  
124 morphometrics were recorded (wing length  $\pm 0.5$ mm with a slotted rule, tarsus length  $\pm 0.1$   
125 mm and head-beak length  $\pm 0.1$  mm with Vernier callipers; Redfern and Clark 2001). The oral  
126 cavity, throat and crop of each bird were also swabbed using an individual sterile viscose  
127 swab, which was then used to inoculate an individual InPouch culture kit (Biomed  
128 Diagnostics, Oregon, USA). Culture kits were incubated at 37°C for 3 – 7 days in order to give  
129 the protozoan parasites sufficient time to culture (Bunbury *et al.* 2005) before isolating  
130 parasites using a standard procedure (further detailed in Lennon *et al.*, 2013). Samples were  
131 then frozen until subsequent analysis.

132

133 In June and July 2012, we also equipped all captured adult Turtle Doves caught with tail-  
134 mounted Pip3 radio-tags (Biotrack, Dorset, UK) weighing 1.7g (<1.5% of body mass), to help  
135 in locating nests. Some of these birds showed clinical symptoms of trichomonosis (see above)  
136 but none appeared lethargic or had any apparent difficulty breathing, and all flew strongly  
137 upon release. Turtle Dove nests were found by monitoring the movements of radio-tagged  
138 birds and cold-searching suitable habitat known to contain territorial males. Nests were  
139 monitored every 2-3 days and when nestlings reached 7 days old, they were ringed, weighed  
140 and were swabbed using the same procedure as for adults. Where nestlings were smaller  
141 than expected (n=2), swabs were taken from the oral cavity only so as not to risk damaging  
142 the oro-pharangeal lining.

143

144 When fresh carcasses of adults (n=2) or nestlings (n=2) were found (i.e. those displaying no  
145 or minimal signs of autolysis), a swab of the oral cavity, throat and crop was taken (as  
146 described above), and any fly eggs or maggots present were removed. The carcasses were  
147 then stored in newspaper and kept at 4°C until gross necropsy could be performed (within 48  
148 hours of being found). A further three nestling carcasses that we couldn't examine post  
149 mortem due to significant fly damage were swabbed for trichomonosis. A moribund Red-  
150 legged Partridge *Alectoris rufa* was also found at one site, and whilst it did not exhibit  
151 diagnostic clinical symptoms of trichomonosis (it was sat in the middle of the farmyard,  
152 unresponsive to stimuli with closed eyes and 'fluffed up' feathers), the bird was retrieved for  
153 necropsy, since it had shared a feeding site with adult Turtle Doves showing clinical signs of  
154 the disease.

155

156 All investigative gross necropsies were carried out by JES following a standard simplified  
157 protocol as previously described (van Riper and van Riper 1980; Cooper 2004; Bunbury *et al.*  
158 2008b) involving both external and internal observation, taking samples from any lesions

159 found for subsequent DNA analysis and the documentation of findings. Clinical signs of  
160 trichomonosis in gross necropsy can include swollen head and eyes and yellow caseous  
161 lesions predominantly found within the oral cavity, pharynx and upper digestive tract (Stabler  
162 1954; Bunbury *et al.* 2008b).

163

164 All carcasses except one were found at the Tolleshunt D'Arcy site in Essex. Thus swabs were  
165 taken from one feeding site and three water sources at this site (stagnant pools in artificial  
166 containers); to determine whether associated food or water sources might be an  
167 environmental source of *T. gallinae* parasites (Kocan 1969).

168

169 Total genomic DNA was extracted from isolated parasites and all trichomonad lesions with a  
170 DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) following the manufacturer's  
171 instructions. DNA extractions were verified with a Nanodrop ND-1000 Spectrophotometer  
172 (Thermo Scientific, Wilmington, USA), to determine DNA concentration.

173

174 An optimised PCR protocol was used with published primers (Gaspar da Silva *et al.* 2007) to  
175 amplify the ITS1/5.8S/ITS2 ribosomal region. PCR reactions were performed in 50 µl volumes  
176 containing 10 µl of extracted DNA with 0.6µM of both primers TFR1 and TFR2, 0.8mM dNTPs,  
177 0.5 units GoTaq Hot Start Polymerase (Promega, Madison, USA), and 1.5mM MgCl<sub>2</sub>. The  
178 thermal profile included an initial denaturation at 94°C for 5 min, then 36 cycles of 94°C for 1  
179 min, 65°C for 30 sec and 72°C for 1 min, and a final extension at 72°C for 5 min. PCR reactions  
180 were run on a GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA) with three  
181 previously identified positives from Columbiformes and one negative control of molecular  
182 water. Each sample was run a total of three times to confirm the presence or absence of  
183 parasites. PCR products were electrophoresed on a 0.8% agarose gel stained with ethidium  
184 bromide in 0.5x TBE buffer. The presence of a 400bp band when amplified products were



185 observed under UV light indicated a positive sample. All positive samples were sequenced by  
186 Source BioScience (Nottingham, UK).

187

188 The ITS1/5.8S/ITS2 ribosomal region of DNA is highly conserved in *Trichomonas* spp., with a  
189 low rate of mutation (Grabensteiner *et al.* 2010), thus any sequences differing in one or more  
190 base pairs were considered to be distinct strains. We used a combination of BioEdit (Hall  
191 2005) and 4Peaks (Griekspoor and Groothuis 2006) to trim, manually align, and assess  
192 forward and reverse sequences for each PCR product for sequencing. As strain length can  
193 influence the closest matching Genbank sequence (authors, pers. obs.), all sequences from this  
194 study were initially aligned with each other in order to identify unique sequences. The longest  
195 of each unique sequence was then queried in the NCBI-BLAST database (Altschul *et al.* 1997).

196

197 To establish whether adults with clinical signs of trichomonosis differed in weight, wing  
198 length, or skeletal measures of size (head-beak length and tarsus length) to apparently  
199 healthy birds, we used general linear models in R (R Core Team 2014). All morphometric  
200 variables were normally distributed, so we designated each in turn as the response variable in  
201 a GLM with gaussian error distributions, and used t values to determine any association  
202 between clinical signs and morphometrics. All birds included in the analysis were adults (i.e.  
203 hatched the previous calendar year or before), with fully grown wings and not in active wing  
204 moult, and we also included in the analysis morphometrics from apparently healthy birds  
205 (that all tested positive for infection by the *T. gallinae* parasite: Lennon *et al.* 2013; Dunn *et al.*  
206 unpublished data) captured during 2011 (n=7) and 2013 (n=14) and measured by JCD. A  
207 subset of birds was subsequently sexed by behavioural observations (through a combination  
208 of observations of purring males, and nest attendance, whereby male Columbiformes incubate  
209 during the middle of the day, and females overnight and during early morning and evening;

210 e.g. Thorsen *et al.* 2004) but we did not include sex in the statistical model due to incomplete  
211 data.  
212

## 213 RESULTS

214 Oral swabs were obtained from 18 adult and seven nestling Turtle Doves during May - July  
215 2012 (n=25; for full details of data collected from each bird see Table 1). In total 13 nests  
216 were monitored, eight of which were depredated prior to hatching. Of the five nests  
217 monitored to nestling stage (full details in Table 1); three nestlings from three nests were  
218 subsequently found dead. *T. gallinae* parasites were cultured from swabs taken from all  
219 nestlings post-mortem, although a full necropsy could only be carried out on two of these due  
220 to the state of decomposition and autolysis. One additional very small nestling (18.9 g  
221 compared to mean weight of  $75.77 \pm 3.82$  g at 7 days (n=11, including data from 2011; Dunn  
222 *et al.* unpublished data) disappeared, and was assumed to have died. A further two nests had  
223 three nestlings between them which were monitored to 7 days old: one nestling was  
224 depredated prior to fledging but the remaining two fledged successfully.

225  
226 Swabs taken from all 25 Turtle Doves tested positive for *T. gallinae*. Of these, three adults  
227 showed clinical signs of trichomonosis, with regurgitated saliva staining the feathering  
228 around the beak. A subset of 12 adults, including two of these clinically affected birds (the  
229 third was caught in May, prior to the start of radio-tagging) were radio-tagged, flew strongly  
230 upon release, and were subsequently relocated. Only the clinically affected birds are  
231 considered further here. Bird 20 (Table 1) was relocated alive on the ground ~90 m from the  
232 capture site at approximately 09:00 on the day following capture (at 19:00). The bird  
233 appeared to be gasping for breath, made no attempt to escape capture by hand and died  
234 shortly afterwards. Bird 21 (Table 1) was relocated ~190 m from the capture site at  
235 approximately 10:00 on the morning following capture (at 16:30). We believe that this bird  
236 was predated as the carcass had been plucked, making it likely that a raptor was responsible.  
237 However, it was impossible to distinguish with certainty between predation and post mortem

238 scavenging. Individuals with clinical signs were lighter and had shorter wings (Table 2),  
239 showing no overlap with non-indicative individuals (Fig 1a). There was no difference in other  
240 skeletal measures of size (Fig 1b; Table 2).

241

242 Gross necropsies were carried out on five independent individuals as detailed in Table 1. Both  
243 Turtle Dove nestlings displayed clinical signs of trichomonosis with a swollen head and eyes  
244 and visible lesions in the buccal cavity and oropharynx (Figures 2a and 2b). One adult female  
245 Turtle Dove was severely emaciated with caseous lesions found blocking the oropharynx  
246 (preventing the bird from swallowing any seed) the location and extent of which can be seen  
247 in Figure 2c. We were unable to suggest cause of death for the second adult turtle carcass  
248 recovered due to the paucity of remains. In contrast to the Turtle Doves examined, the Red-  
249 legged Partridge had no visible lesions within the buccal cavity or upper respiratory tract,  
250 although an oral swab taken from the dead bird tested positive for *T. gallinae* parasites. A  
251 caseous trichomonosis lesion was found to have originated within the proventriculus, grown  
252 through the wall and fused to a lobe of the liver resulting in the necrosis of the connecting  
253 tissue and discolouration (Figure 2d).

254

255 Sequences in both directions were obtained from the 25 individuals screened; however,  
256 sequence quality from 6 individuals was too poor to give meaningful data (Table 1). Two  
257 identical sequences were obtained from lesions and oral swabs from three individuals (IDs  
258 20, 22 and 26: Table 1). Overall four distinct sequences were obtained; the most common  
259 sequence (JN007005.1: 100% query coverage and 100% max identity) was isolated from 16  
260 individuals, including all birds displaying clinical signs, all dead Turtle Doves (adults and  
261 nestlings), and the Red-legged Partridge (Table 1). Three sequences were isolated from water  
262 sources and one sequence from a feed site, which all matched Genbank sequence JN007005.1  
263 (100% query coverage and 100% max identity; Table 1). Sequences from two individuals

264 matched sequence FN433475.1 (100% query coverage and 100% max identity), and  
265 sequences isolated from one individual each matched Genbank sequences AJ784785.1 (99%  
266 query coverage and 98% max identity) and FN433473.1 (99% query coverage and 100% max  
267 identity; Table 1).

268

269 **DISCUSSION**

270 We report probably the first confirmation of mortality in free-living Turtle Doves with clinical  
271 signs of trichomonosis. We found a 100% rate of infection by *T. gallinae* in the 25 live Turtle  
272 Doves screened during 2012. This is higher than during the previous year (n=14; Lennon *et al.*  
273 2013), and combined with previous data gives an overall infection rate of 95% (n=39) across  
274 sites separated by up to 120 km. The only two individuals apparently negative for *T. gallinae*  
275 infection were two nestlings from the same nest in 2011 (Lennon *et al.* 2013). Whilst two of  
276 our nests were related to infected but non indicative adults the remaining three nests that  
277 reached the nestling stage were independent of all adults sampled. Thus, only three nestlings  
278 with clinical signs were related to any of the adults caught; and no adults with clinical signs  
279 were related to any nests.

280

281 The overall rate of *T. gallinae* infection appear unusually high when compared to other  
282 Columbidae (e.g. 19% in Spotted Dove *Streptopelia chinensis* and 59% in Zebra Dove *Geopelia*  
283 *straita*, Bunbury *et al.* 2007; 5.6% in Mourning Doves *Zenaida macroura*, Schulz *et al.* 2005;  
284 34.2% in wintering Wood Pigeons *Columba palumbus*, Villanúa *et al.* 2006), with only Rock  
285 Pigeons *Columba livia* documented as having similarly high rates of infection (92%: Sansano-  
286 Maestre *et al.* 2009). Sub-clinical infection can impact on survival: for example, Pink Pigeons  
287 testing positive for *T. gallinae* infection were 13% less likely to survive for a further two years  
288 after screening than those testing negative (Bunbury *et al.* 2008a). Usually, only a very small  
289 percentage of individuals infected by *T. gallinae* display clinical signs (e.g. 0.37% of  
290 Columbidae, Sansano-Maestre *et al.* 2009; 1.9% of Pink Pigeons, Bunbury *et al.* 2008a).  
291 However, we report clinical signs in 28% of individuals infected by *T. gallinae* parasites (three  
292 adults and four nestlings).

293

294 All fatal cases of trichomonosis were linked to the same strain of *T. gallinae* found at our study  
295 sites in both Turtle Doves and Woodpigeons (Lennon *et al.* 2013), which was also isolated  
296 from the only Turtle Dove showing clinical signs during 2011 (a nestling that was predated  
297 prior to fledging; Lennon *et al.* 2013). This strain falls within the same clade as *T. gallinae*  
298 strain A (Lawson *et al.* 2011a; Lennon *et al.* 2013; Chi *et al.* 2013) and is identical at the  
299 ITS/5.8S/ITS2 region to the causative agent of the finch trichomonosis epizootic (Robinson *et*  
300 *al.* 2010; Ganas *et al.* 2014). As we only sequenced the ITS/5.8S/ITS2 region, we acknowledge  
301 that we may be observing more than one strain that is genetically different at other functional  
302 genes. The clade contains strains found in Columbidae worldwide, raptors in Spain, and  
303 finches in the USA and UK, suggesting inter- and intra-specific transmission. Further PCR  
304 work is required to determine whether or not this strain is identical to the epizootic strain  
305 reported in finches (Robinson *et al.* 2010; Lawson *et al.* 2011), by examining other functional  
306 genes such as the iron hydrogenase gene (Lawson *et al.* 2011a; Lennon *et al.* 2013).

307

308 Necropsies carried out on intact Turtle Dove carcasses (one adult, two nestlings) strongly  
309 suggested trichomonosis as the cause of death and identified large oropharyngeal lesions.  
310 Molecular testing of DNA extracted from the lesions confirmed the gross necropsy diagnoses.  
311 Adult 20 was severely emaciated, but in contrast adult 21 had substantial muscle reserves  
312 over the sternum suggesting that this bird might have been at an earlier stage of infection,  
313 although the paucity of remains did not allow us to establish this with any certainty. The  
314 observation of clinical trichomonosis in adult and nestling Turtle Doves is, to our knowledge,  
315 the first suggestion of mortality associated with trichomonosis caseous lesions in this species.  
316 Whilst we did not screen for other pathogens and cannot rule out the possibility of co-  
317 infection increasing susceptibility to *T. gallinae*, the final cause of death was believed to be  
318 due to *T. gallinae* lesions. Controlled experimental infections in the absence of co-infecting  
319 pathogens would be necessary to confirm trichomonosis as causing mortality.

320

321 That individuals showing clinical signs of disease were considerably lighter than those  
322 without is not unexpected: *T. gallinae* lesions constrict the oesophagus and prevent affected  
323 birds from ingesting food, resulting in decreased weight. However, the difference in wing  
324 lengths is marked, with no overlap between the wing lengths of individuals with and without  
325 clinical signs, and a mean 3.47% reduction in the wing length of individuals with clinical signs  
326 compared to those without. Our sample size of birds showing clinical signs is small, and thus  
327 our results should be treated with some caution. There were no differences in any skeletal  
328 measures of size, suggesting that infection may impact upon wing length during moult on  
329 wintering grounds in Africa through competition for energetic resources, rather than smaller  
330 birds simply being more susceptible to infection. Such a mechanism has been proposed  
331 previously in other host-parasite systems, with *Haemoproteus* and *Plasmodium* spp. (Marzal *et al.*  
332 *et al.* 2013), *Haemoproteus* spp. (Dunn *et al.* 2013), *Leucocytozoon* spp. (Hatchwell *et al.* 2001)  
333 and *Trypanosoma* spp. (Rätti *et al.* 1993) posited to reduce feather length through competition  
334 for host resources during moult. Turtle Doves are Europe's only trans-Saharan migrant  
335 Columbidae and undergo a partial post-breeding moult prior to migration, completing their  
336 moult on the African wintering grounds (Baker 1993). Thus, individuals with clinical signs  
337 during summer 2012 may have acquired infections on, or en route to/from, their wintering  
338 grounds, or even during the previous breeding season, highlighting the need to further  
339 understand the dynamics of *T. gallinae* infection throughout the annual cycle of migratory  
340 species.

341

342 The finding of a moribund Red-legged Partridge, and subsequent suggestion of the same  
343 strain of *T. gallinae* causing markedly different pathology (through isolation of the parasite  
344 from the lesion) is interesting. Previous work had discounted the possibility of parasite spill  
345 over between Columbidae and introduced Galliformes such as Red-legged Partridges and



346 common Pheasants *Phasianus colchicus* (Lennon *et al.* 2013), as Galliformes tend to be  
347 infected by *T. gallinarum*, which is genetically distinct from *T. gallinae* (e.g Pennycott 1998).  
348 However, our findings suggest that such a parasite spill over may potentially occur. This  
349 suggests that screening of Galliformes may be worthwhile in order to establish whether  
350 parasite spill over between Columbidae and Galliformes – and potentially Passerines - is a  
351 possible occurrence at shared food resources such as game bird feeders or grain spills in  
352 farmyards. Such parasite transfer may occur potentially through a similar mechanism to that  
353 suggested by Lawson *et al.* (2012) for the putative parasite spill over from Columbidae to  
354 Passerines.

355  
356 The same predominant single strain of *T. gallinae* isolated from the moribund Turtle Doves  
357 and Red-legged Partridge was also isolated from both a farmyard grain pile and three artificial  
358 water sources at one of our sites. Food and water sources have previously been postulated as  
359 potential vectors for transfer of *T. gallinae* parasites (Kocan 1969), although Bunbury *et al.*  
360 (2007) found no positive grain samples, and only 2 out of 15 water samples to be positive for  
361 trichomonads. Whilst speculative, the unusually wet summer of 2012 may have allowed  
362 parasites to survive for longer on damp grain piles (Kocan 1969; Erwin *et al.* 2000) meaning  
363 that individual birds may have been subjected to high and repeated doses of *T. gallinae*  
364 parasites from repeat visits to infected food and water sources. Further work should examine  
365 the survival of parasites in food and water sources in these settings to gauge natural infection  
366 rates in relation to the density of potential hosts, and weather-related factors.

367  
368 Turtle Dove populations in NW Europe have been declining for decades and continue to do so.  
369 Whilst a previous intensive study of this species on UK breeding grounds found no evidence of  
370 disease-related issues (S. Browne, pers. comm.), no historic data on infection rates are  
371 available. The species has also undergone a dietary switch in the UK, from the seeds of arable

372 plants (Murton *et al.* 1964) to anthropogenic seed resources such as grain piles in farmyards  
373 (Browne and Aebischer 2003). Food stress can decrease immune function (Lindström *et al.*  
374 2005) and induce chronic stress in birds (Clinchy *et al.* 2004), potentially increasing  
375 susceptibility to infection and the likelihood of clinical signs and this possibility cannot be  
376 negated within this system. More likely, however, is that the dietary switch undergone by this  
377 species has led to an increased risk of intra- and inter-species transference of directly and  
378 indirectly-transmitted parasites and pathogens, such as *T. gallinae*, at a restricted number of  
379 food resources shared by birds feeding at high densities (e.g. Höfle *et al.* 2004; Lawson *et al.*  
380 2012).

381

382 Historically, the anti-protozoal dimetridazole, or Emtryl, was widely used as a prophylactic  
383 feed additive for game birds reared for sporting purposes, however, since its withdrawal in  
384 2002 concerns have been raised about the potential impacts of motile protozoans on a wide  
385 range of species, mostly captive-reared birds (Dernburg *et al.* 2005; Callait-Cardinal *et al.*  
386 2007). To our knowledge, no literature is available examining any trends in infection rates of  
387 trichomonads in captive-reared game birds during the period since Emtryl withdrawal,  
388 although Lennon *et al.* (2013) found higher rates of trichomonad infection in Columbidae on  
389 farms with game bird feeding than on farms without, and Höfle *et al.* (2004) suggest that the  
390 supplementary feeding of game birds constitutes a risk factor for the appearance of  
391 trichomonosis outbreaks in wild birds. We suggest that the potential for parasite transfer  
392 from non-native game birds to rapidly declining native species is worthy of further  
393 investigation. Supplementary feeding of game and wild birds, especially during the late winter  
394 period when seed food is scarce, is widespread. Although turtle doves are summer migrants  
395 and therefore not present in Europe during the winter, given the results presented here, and  
396 the recent finch trichomonosis epizootic (Robinson *et al.* 2010), we suggest stringent hygiene  
397 precautions when deploying supplementary food are needed throughout the year to reduce

398 the risk of disease transmission. These include strict adherence to guidelines to only  
399 distribute enough food to match consumption, ensure a fresh supply of food is maintained  
400 without leaving seed unconsumed and rotating feeding sites. (e.g. Natural England 2012).  
401  
402 Our work highlights the importance of continued monitoring of *T. gallinae* infection in Turtle  
403 Doves and of monitoring sub-clinical infection in free-living populations rather than relying  
404 on morbidity and mortality reports alone, particularly for species where the population status  
405 gives cause for conservation concern. Further work should address the epidemiology of *T.*  
406 *gallinae* infection, as well as establishing any sub-clinical impacts of infection that may impact  
407 on ecological parameters such as reproductive success. *T. gallinae* is thought to be a  
408 population-limiting factor in the Pink Pigeon, despite observed pathogenicity being low  
409 (Bunbury *et al.* 2008a). Unless Turtle Dove feeding ecology changes to allow a reduction in  
410 infection rates, parasite infection may potentially amplify the existing reduction in  
411 reproductive output and either hasten the ongoing population decline or prevent population  
412 recovery. Greater uptake of measures that provide abundant and accessible food (e.g. fallows,  
413 seed mixes or cultivated, uncropped margins), which are available in many European agri-  
414 environment schemes, would provide birds with more dispersed feeding opportunities and  
415 thus potentially reduce disease transmission.

416

## 417 **ACKNOWLEDGEMENTS**

418 Catching and ringing was carried out under licence from the British Trust for Ornithology and  
419 sampling for *T. gallinae* was carried out under license from the Home Office. We thank Jacqui  
420 Weir and Kerry Skelhorn for their help radio-tracking birds and retrieving corpses. Finally, we  
421 thank four anonymous reviewers whose comments greatly improved an earlier draft of the  
422 manuscript.

423

424 **FINANCIAL SUPPORT**

425 This work was funded jointly by the RSPB and Natural England as part of the *Action for Birds*  
426 *in England* (AfBiE) partnership.

427

428   **REFERENCES**

- 429   **Altschul, S.F., Madden, T.L., Schäffer, A.A., Zhang, J., Zhang, Z., Miller, W. and Lipman, D.J.** (1997)  
430       Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic*  
431       *Acids Research*, **25**, 3389–3402. doi: 10.1093/nar/25.17.3389
- 432   **Amin, A., Bilic, I., Liebhart, D. and Hess, M.** (2014) Trichomonads in birds - a review. *Parasitology*,  
433       **141**, 733–47. doi: 10.1017/S0031182013002096
- 434   **Baker, K.** (1993) *Identification Guide to European Non-Passerines. BTO Guide 24*. British Trust for  
435       Ornithology, Thetford.
- 436   **Balmer, D.E., Gillings, S., Caffrey, B., Swann, B., Downie, I. and Fuller, R.** (2013) *Bird Atlas 2007-*  
437       *11*.
- 438   **BonDurant, R. and Honigberg, B.** (1994) Trichomonads of veterinary importance. *Parasitic*  
439       *Protozoa* (ed J. Kreier), pp. 111–206. Academic Press, New York.
- 440   **Browne, S. and Aebischer, N.** (2003) Habitat use, foraging ecology and diet of Turtle Doves  
441       *Streptopelia turtur* in Britain. *Ibis*, **145**, 572–582. doi: 10.1046/j.1474-919X.2003.00185.x.
- 442   **Browne, S.J. and Aebischer, N.** (2004) Temporal changes in the breeding ecology of European  
443       Turtle Doves *Streptopelia turtur* in Britain, and implications for conservation. *Ibis*, **146**, 125–  
444       137. doi: 10.1111/j.1474-919X.2004.00235.x
- 445   **Bunbury, N., Bell, D., Jones, C., Greenwood, A. and Hunter, P.** (2005) Comparison of the InPouch  
446       TF culture system and wet-mount microscopy for diagnosis of *Trichomonas gallinae* infections in  
447       the pink pigeon *Columba mayeri*. *Journal of Clinical Microbiology*, **43**, 1005–1006. doi:  
448       10.1128/JCM.43.2.1005-1006.2005.

449 **Bunbury, N., Jones, C.G., Greenwood, A.G. and Bell, D.J.** (2007) *Trichomonas gallinae* in Mauritian  
450 columbids: implications for an endangered endemic. *Journal of Wildlife Diseases*, **43**, 399–  
451 407.

452 **Bunbury, N., Jones, C.G., Greenwood, A.G. and Bell, D.J.** (2008a) Epidemiology and conservation  
453 implications of *Trichomonas gallinae* infection in the endangered Mauritian Pink Pigeon.  
454 *Biological Conservation*, **141**, 153–161. doi: 10.1016/j.biocon.2007.09.008.

455 **Bunbury, N., Stidworthy, M.F., Greenwood, A.G., Jones, C.G., Sawmy, S., Cole, R.E., Edmunds, K.**  
456 **and Bell, D.J.** (2008b) Causes of mortality in free-living Mauritian pink pigeons *Columba*  
457 *mayeri*, 2002-2006. *Endangered Species Research*, **9**, 213–220.

458 **Callait-Cardinal, M.-P., Leroux, S., Venereau, E., Chauve, C.M., Le Pottier, G. and Zenner, L.**  
459 (2007) Incidence of histomonosis in turkeys in France since the bans of dimetridazole and  
460 nifursol. *Veterinary Record*, **161**, 581–585. doi: 10.1136/vr.161.17.581

461 **Chi, J.F., Lawson, B., Durrant, C., Beckmann, K., John, S., Alrefaei, A.F., Kirkbride, K., Bell, D.J.,**  
462 **Cunningham, A. a and Tyler, K.M.** (2013) The finch epidemic strain of *Trichomonas gallinae* is  
463 predominant in British non-passerines. *Parasitology*, **140**, 1234–45. doi:  
464 10.1017/S0031182013000930

465 **Clinchy, M., Zanette, L., Boonstra, R., Wingfield, J.C. and Smith, J.N.M.** (2004) Balancing food and  
466 predator pressure induces chronic stress in songbirds. *Proceedings of the Royal Society B:*  
467 *Biological Sciences*, **271**, 2473–2479. doi: 10.1098/rspb.2004.2913.

468 **Cooper, J.** (2004) Information from dead and dying birds. *Bird ecology and conservation: a*  
469 *handbook of techniques* (eds W. Sutherland, I. Newton and R. Green), pp. 179–209. Oxford  
470 University Press, Oxford.

471 **Dernburg, A., Rogier-Saderne, M.-C., Chauve, C. and Zenner, L.** (2005) Consequences of the  
472 withdrawal of dimetridazole on intestinal parasitism in ducks. *Veterinary Record*, **156**, 148–  
473 150. doi:10.1136/vr.156.5.148

474 **Dunn, J.C., Goodman, S.J., Benton, T.G. and Hamer, K.C.** (2013) Avian blood parasite infection  
475 during the non-breeding season: an overlooked issue in declining populations? *BMC Ecology*,  
476 **13**, 30. doi:10.1186/1472-6785-13-30

477 **Eaton, M., Cuthbert, R., Dunn, E., Grice, P., Hall, C., Hayhow, D., Hearn, R., Holt, C., Knipe, A.,**  
478 **Marchant, J., Mavor, R., Moran, N., Mukhida, F., Musgrove, A., Noble, D., Oppel, S., Risely,**  
479 **K., Stroud, D., Toms, M. and Wotton, S.** (2012) *The State of the UK's Birds 2012*. Sandy,  
480 Bedfordshire.

481 **Erwin, K.G., Kloss, C., Lyles, J., Felderhoff, J., Fedynich, A.M., Henke, S.E. and Roberson, J.A.**  
482 (2000) Survival of *Trichomonas gallinae* in white-winged dove carcasses. *Journal of Wildlife*  
483 *Diseases*, **36**, 551–554. doi: 10.7589/0090-3558-36.3.551

484 **Forrester, D.J. and Foster, G.W.** (2008) Trichomonosis. *Parasitic Diseases of Wild Birds* (eds C.T.  
485 Atkinson, N.J. Thomas and D.B. Hunter), pp. 120–153. Ames, IA, USA.

486 **Ganas, P., Jaskulska, B., Lawson, B., Zadavec, M., Hess, M. and Bilic, I.** (2014) Multi-locus  
487 sequence typing confirms the clonality of *Trichomonas gallinae* isolates circulating in  
488 European finches. *Parasitology*, **141**, 652–61. doi: 10.1017/S0031182013002023

489 **Gaspar da Silva, D., Barton, E., Bunbury, N., Lunness, P., Bell, D.J. and Tyler, K.M.** (2007)  
490 Molecular identity and heterogeneity of Trichomonad parasites in a closed avian population.  
491 *Infection, Genetics and Evolution*, **7**, 433–440. doi: 0.1016/j.meegid.2007.01.002.

492 **Grabensteiner, E., Bilic, I., Kolbe, T. and Hess, M.** (2010) Molecular analysis of clonal trichomonad  
493 isolates indicate the existence of heterogenic species present in different birds and within the  
494 same host. *Veterinary Parasitology*, **172**, 53–64. doi:10.1016/j.vetpar.2010.04.015. doi:  
495 10.1016/j.vetpar.2010.04.015

496 **Griekspoor, A. and Groothuis, T.** (2006) 4 Peaks. Version 1.7.2. mekentosj.com.

497 **Hall, T.** (2005) BioEdit: biological sequence alignment editor for Win95/98/NT/2K/XP. Ibis  
498 Biosciences, Carlsbad, CA, USA. <http://www.mbio.ncsu.edu/BioEdit/bioedit>.

499 **Hatchwell, B.J., Wood, M.J., Anwar, M.A., Chamberlain, D. and Perrins, C.** (2001) The  
500 haematozoan parasites of Common Blackbirds *Turdus merula*: associations with host  
501 condition. *Ibis*, **143**, 420–426.

502 **Höfle, U., Gortazar, C., Ortíz, J.A., Knispel, B. and Kaleta, E.F.** (2004) Outbreak of trichomoniasis in  
503 a woodpigeon (*Columba palumbus*) wintering roost. *European Journal of Wildlife Research*, **50**, 73–  
504 77. doi:10.1007/s10344-004-0043-2..

505 **Kocan, R.** (1969) Various grains and liquid as potential vehicles of transmission of *Trichomonas*  
506 *gallinae*. *Bulletin of the Wildlife Disease Association*, **5**, 148–149.

507 **Krone, O., Altenkamp, R. and Kenntner, N.** (2005) Prevalence of *Trichomonas gallinae* in northern  
508 goshawks from the Berlin area of northeastern Germany. *Journal of Wildlife Diseases*, **41**,  
509 304–309. doi: 10.7589/0090-3558-41.2.304

510 **Lawson, B., Cunningham, A.A., Chantrey, J., Hughes, L.A., John, S.K., Bunbury, N., Bell, D.J. and**  
511 **Tyler, K.M.** (2011a) A clonal strain of *Trichomonas gallinae* is the aetiologic agent of an emerging  
512 avian epidemic disease. *Infection, Genetics and Evolution*, **11**, 1638–1645. doi:



513 10.1016/j.meegid.2011.06.007.

514 **Lawson, B., Robinson, R.A., Colvile, K.M., Peck, K.M., Chantrey, J., Pennycott, T.W., Simpson,**  
515 **V.R., Toms, M.P. and Cunningham, A.A.** (2012) The emergence and spread of finch  
516 trichomonosis in the British Isles. *Philosophical transactions of the Royal Society of London B*,  
517 **367**, 2852–2863. doi: 10.1098/rstb.2012.0130..

518 **Lawson, B., Robinson, R.A., Neimanis, A., Handeland, K., Isomursu, M., Agren, E.O., Hamnes, I.S.,**  
519 **Tyler, K.M., Chantrey, J., Hughes, L.A., Pennycott, T.W., Simpson, V.R., John, S.K., Peck,**  
520 **K.M., Toms, M.P., Bennett, M., Kirkwood, J.K. and Cunningham, A.A.** (2011b) Evidence of  
521 spread of the emerging infectious disease, finch trichomonosis, by migrating birds. *EcoHealth*,  
522 **8**, 143–53. doi: 10.1007/s10393-011-0696-8

523 **Lehikoinen, A., Lehikoinen, E., Valkama, J., Väisänen, R. and Isomursu, M.** (2013) Impacts of  
524 trichomonosis epidemics on Greenfinch *Chloris chloris* and Chaffinch *Fringilla coelebs*  
525 populations in Finland. *Ibis*, **155**, 357–366. doi: 10.1111/ibi.12028

526 **Lennon, R.J., Dunn, J.C., Stockdale, J.E, Goodman, S.J., Morris, A.J. and Hamer, K.C.** (2013)  
527 Trichomonad parasite infection in four species of Columbidae in the UK. *Parasitology*, **140**,  
528 1368–1376. doi: 10.1017/S0031182013000887

529 **Lindström, K.M., Hawley, D.M., Davis, A.K. and Wikelski, M.** (2005) Stress responses and disease  
530 in three wintering house finch (*Carpodacus mexicanus*) populations along a latitudinal gradient.  
531 *General and Comparative Endocrinology*, **143**, 231–239. doi: 10.1016/j.

532 ygcen.2005.04.005.

533 **Marzal, A., Asghar, M., Rodríguez, L., Reviriego, M., Hermosell, I.G., Balbontín, J., Garcia-**  
534 **Longoria, L., de Lope, F. and Bensch, S. (2013)** Co-infections by malaria parasites decrease  
535 feather growth but not feather quality in house martin. *Journal of Avian Biology*, **44**, 437–444.  
536 doi: 10.1111/j.1600-048X.2013.00178.x

537 **Muñoz, E. (1995)** *Estudio de La Prevalencia Y Susceptibilidad a La Infección Por Trichomonas*  
538 *Gallinae En Aves Domésticas Y Silvestres. Valoración de La Sensibilidad Del Protozoo a*  
539 *Diferentes Derivados Imidazólicos*. Universitat Autònoma de Barcelona, Cerdanyola,  
540 Catalonia, Spain.

541 **Murton, R.K., Westwood, N.J. and Isaacson, A. (1964)** The feeding habits of the Woodpigeon  
542 *Columba palumbus*, Stock Dove *C. oenas* and Turtle Dove *Streptopelia turtur*. *Ibis*, **106**, 174–  
543 188. doi: 10.1111/j.1474-919X.1964.tb03694.x

544 **Natural England. (2012)** *Entry Level Stewardship: Environmental Stewardship Handbook. Fourth*  
545 *Edition - January 2013*. Natural England.

546 **PECBMS. (2012)** *Population Trends of Common European Breeding Birds*. Prague.

547 **Pennycott, T.W. (1998)** Carriage of trichomonads, Hexamita species and Blastocystis species by  
548 adult pheasants. *Veterinary record*, **143**, 142–143 doi: 10.1136/vr.143.5.142.

549 **R Core Team. (2012)** R: A Language and Environment for Statistical Computing. R Foundation for  
550 Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0. www.R-project.org.

551 **Rätti, O., Dufva, R. and Alatalo, R. (1993)** Blood parasites and male fitness in the Pied Flycatcher.  
552 *Oecologia*, **96**, 410–414. doi: 10.1007/BF00317512

553 **Redfern, C. and Clark, J. (2001)** *Ringers' Manual*. British Trust for Ornithology, Thetford.

554 **Van Riper, C. and van Riper, S.** (1980) A necropsy procedure for sampling disease in wild birds.  
555 *The Condor*, **82**, 85–98.

556 **Robinson, R.A., Lawson, B., Toms, M.P., Peck, K.M., Kirkwood, J.K., Chantrey, J., Clatworthy, I.R.,**  
557 **Evans, A.D., Hughes, L.A., Hutchinson, O.C., John, S.K., Pennycott, T.W., Perkins, M.W., Rowley,**  
558 **P.S., Simpson, V.R., Tyler, K.M. and Cunningham, A.A.** (2010) Emerging infectious disease leads to  
559 rapid population declines of common British birds. *PloS One*, **5**, e12215. doi:  
560 10.1371/journal.pone.0012215.

561 **Sansano-Maestre, J., Garijo-Toledo, M.M. and Gómez-Muñoz, M.T.** (2009) Prevalence and  
562 genotyping of *Trichomonas gallinae* in pigeons and birds of prey. *Avian Pathology*, **38**, 201–  
563 207.

564 **Schulz, J.H., Bermudez, A.J. and Millspough, J.J.** (2005) Monitoring presence and annual variation  
565 of Trichomoniasis in Mourning Doves. *Avian Diseases*, **49**, 387–389.

566 **Stabler, R.** (1954) *Trichomonas gallinae*: a review. *Experimental parasitology*, **3**, 368–402.

567 **Thorsen, M., Innes, J., Nugent, G. and Prime, K.** (2004) Parental care and growth rates of New  
568 Zealand pigeon (*Hemiphaga novaeseelandiae*) nestlings. *Notornis*, **51**, 136–140.

569 **Villanúa, D., Höfle, U., Pérez-Rodríguez, L. and Gortázar, C.** (2006) *Trichomonas gallinae* in  
570 wintering common wood pigeons *Columba palumbus* in Spain. *Ibis*, **148**, 641–648. doi:  
571 10.1111/j.1474-919X.2006.00561.x.

572

573

574

575 Table 1. Summary of data collected from individual birds.

ID	Outcome	Species	Age	<i>T. gallinae</i> source <sup>a</sup>	Post mortem	Genbank match
1- 16	Live	Turtle Dove	Adults	1	No	JN007005.1 (n=8) FN433475.1 (n=1) FN433473.1 (n=1) AJ784785.1 (n=1) No sequence (n=5)
17 - 18	Live	Turtle Dove	Nestling (nest 1)	1	No	JN007005.1 (n=1) FN433475.1 (n=1)
19	Predated	Turtle Dove	Nestling (nest 2)	2	No	JN007005.1
20	Died	Turtle Dove	Adult	1, 4	Yes	JN007005.1
21	Predated/Died	Turtle Dove	Adult	1	Yes	JN007005.1
22	Died	Turtle Dove	Nestling (nest 3)	3, 4	Yes	JN007005.1
23	Disappeared (assumed died)	Turtle Dove	Nestling (nest 3)	2	No	No sequence
24	Died	Turtle Dove	Nestling (nest 4)	3	No	JN007005.1
25	Died	Turtle Dove	Nestling (nest 5)	3	Yes	JN007005.1
26	Died	Red legged partridge	Adult	3 ,4	Yes	JN007005.1

576

577 <sup>a</sup> *T. gallinae* source: 1: swab collected from crop, throat and oral cavity whilst alive; 2: swab  
578 collected from oral cavity only; 3: swab collected post mortem; 4: DNA extracted directly from  
579 lesion.

580

581

582 Table 2. Summary of morphometrics for adult Turtle Doves with and without clinical signs of  
583 trichomonosis.

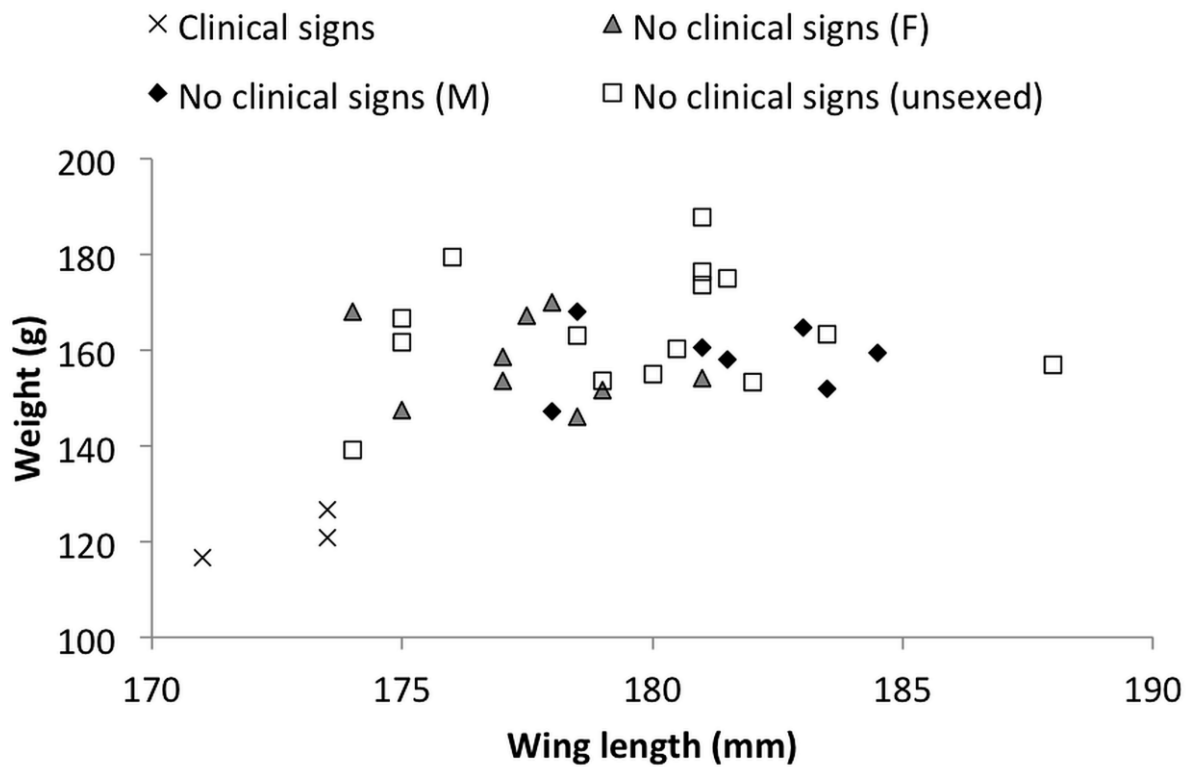
Measurement	Mean $\pm$ 1 SE		Statistics		
	Clinical signs	No clinical signs	t	df	p
	(n=3)	(n=31)			
<b>Weight (g)</b>	<b>121.40 <math>\pm</math> 2.93</b>	<b>161.06 <math>\pm</math> 1.92</b>	<b>-6.276</b>	<b>1</b>	<b>&lt;0.001</b>
<b>Wing length (mm)</b>	<b>172.67 <math>\pm</math> 0.83</b>	<b>179.45 <math>\pm</math> 0.59</b>	<b>-3.493</b>	<b>1</b>	<b>0.001</b>
Head-beak length (mm)	46.57 $\pm$ 0.92	46.23 $\pm$ 0.15	0.623	1	0.538
Tarsus length (mm)	23.23 $\pm$ 1.17	23.52 $\pm$ 0.19	-0.416	1	0.680

584

585

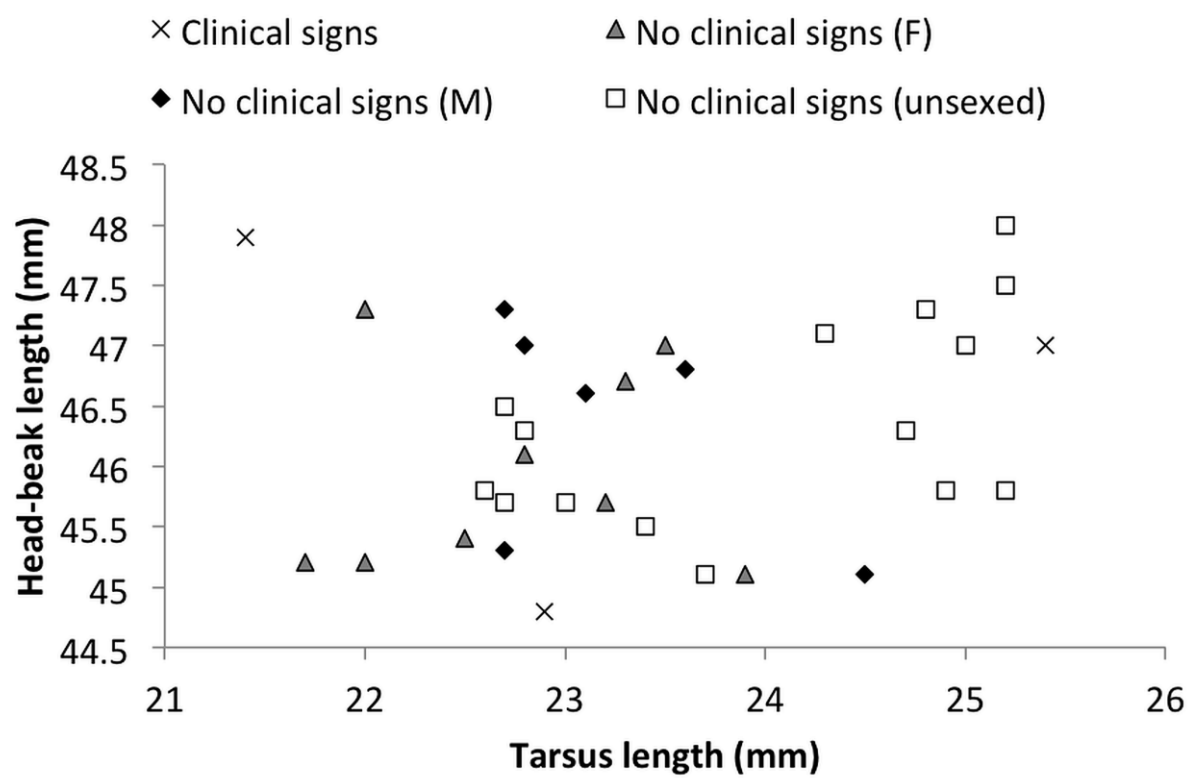
586 Figure 1. a) Wing length and weight distributions and b) head-beak and tarsus length  
587 distributions from adult turtle doves with clinical signs compared to female, male and  
588 unsexed adults with no clinical signs.

589 a)

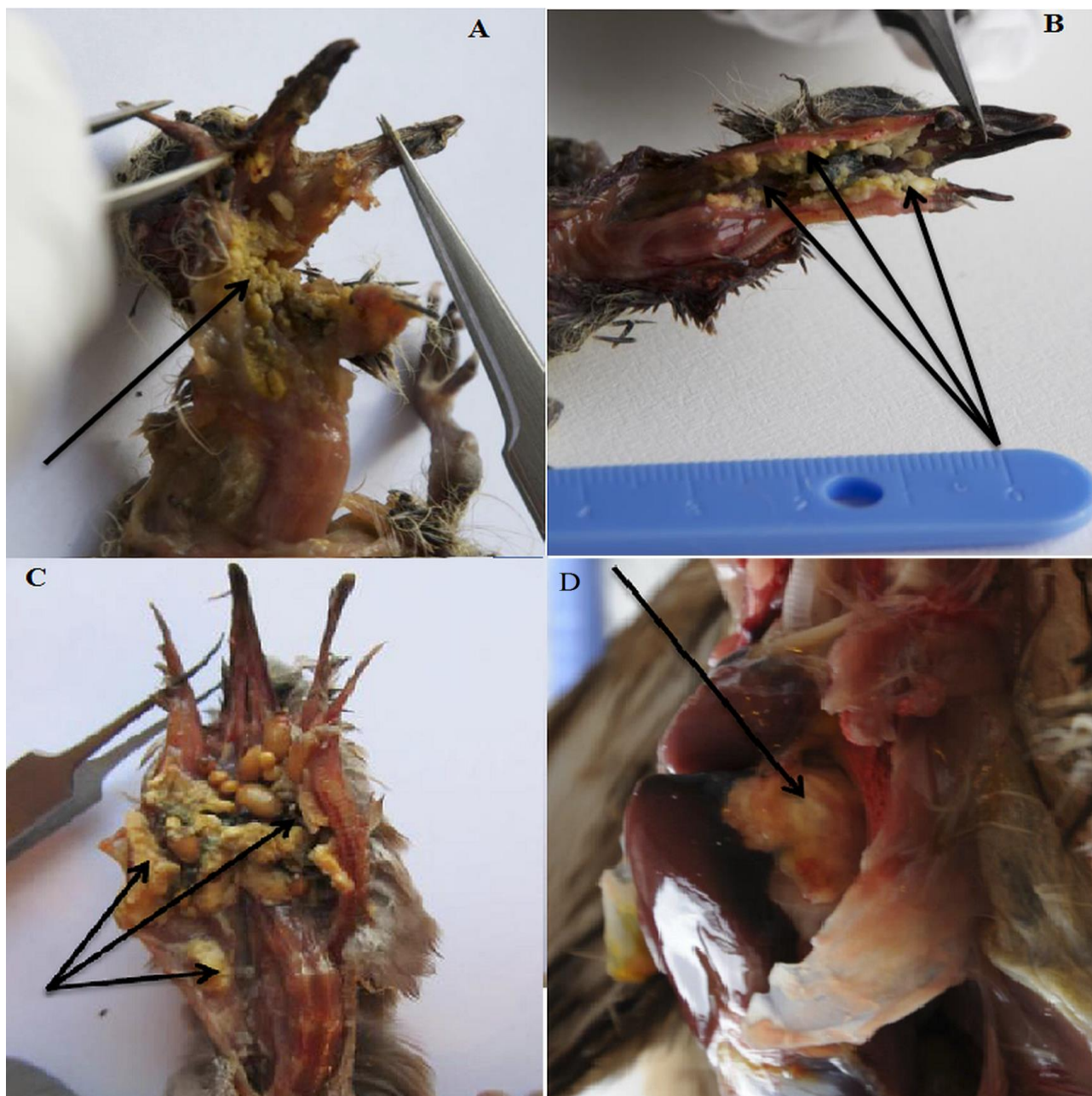


590

591 b)



593 Figure 2. Photographs from post-mortems of A) nestling Turtle Dove 25, B) nestling Turtle  
594 Dove 22, C) adult Turtle Dove 20, and D) Red-legged Partridge 26. Arrows show  
595 oropharyngeal lesions in Turtle Doves and a lesion originating in the proventriculus in the  
596 Red-legged Partridge.



597